ERDC/CERL TR-09-34

Construction Engineering
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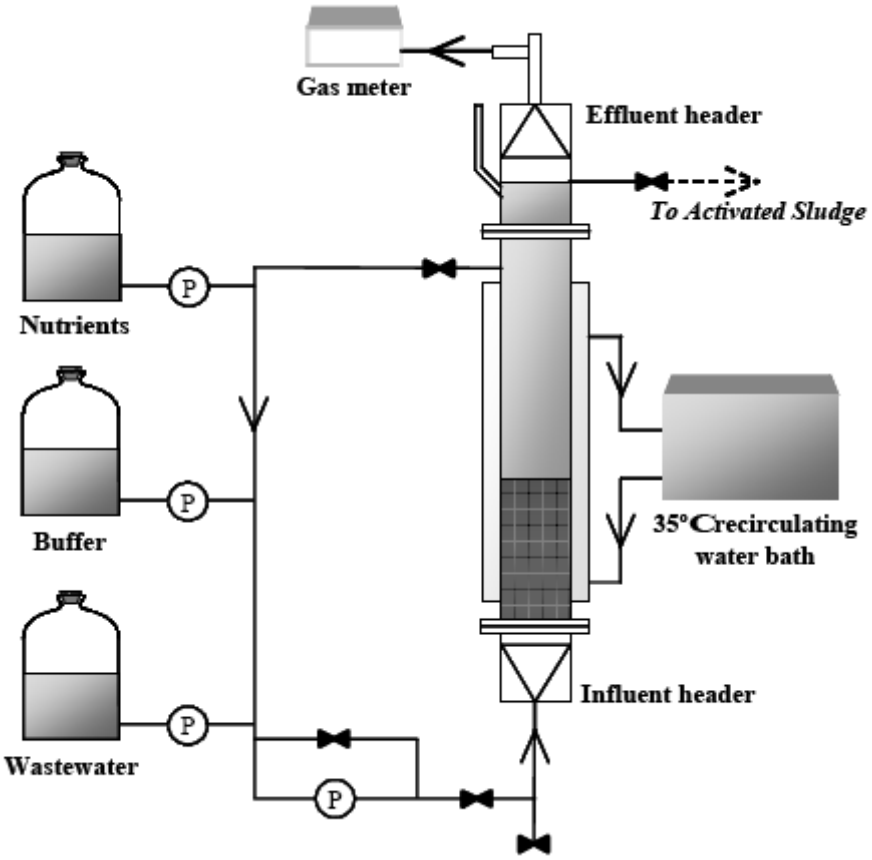
Polymerase Chain Reaction (PCR) Analysis of Microbial Consortia in Wastewater Treatment Processes for High Explosives

Clint M. Arnett, Giselle Rodriguez, and Stephen W. Maloney

September 2009



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Construction Engineering Research Laboratory (CERL)
U.S. Army Engineer Research and Development Center
2902 Newmark Dr.
Champaign, IL. 61822-1076

Final Report

Prepared for Headquarters, U.S. Army Corps of Engineers
Washington, DC 20314-1000

Under Work Unit 120190

Abstract: The engineering aspects of bioreactors treating wastewaters contaminated with high explosives are better understood than the microbial communities within the reactors. Five anaerobic bioreactors treating energetics were studied to better understand bacterial compositions in each reactor system as related to influents containing various energetic materials. Studied were: three anaerobic fluidized bed reactors (AFBR) treating DNAN and MNA; one gas lift bioreactor (GLBR) treating ammonium perchlorate; and one pilot-scale granulated activated carbon-fluidized bed reactor (GAC-FBR) treating TNT, RDX, and TNB. Bacterial profiles were derived by cloning and sequencing the total 16S rRNA genes from each reactor contaminant combination. In the presence of DNAN and MNA, the AFBRs communities showed bacteria of the phylum *Chloroflexi* represented 30 percent of the total clone distribution in each reactor. In the presence of ammonium perchlorate, the GLBR was composed primarily of *Proteobacteria* in excess of 60 percent of the clone distribution. The GAC-FBR treating TNT, RDX, and TNB was enriched in both *Chloroflexi* and *Proteobacteria* (37 and 27 percent of the total clone distribution, respectively). Assuming the most prevalent bacteria were enriched by the presence of the contaminant, the data indicated that *Chloroflexi* and *Proteobacteria* likely played an important roll in energetic degradation.

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Preface

This work was performed for U.S. Army Engineering Research and Development Center (ERDC) 6.2 Applied Research Program. The Work Package tile: *Mission Sustainment in the Industrial Base for Insensitive Munition Compounds*. Project title: *Microbial Consortia Variation by High Explosive Component Feed*.

This work was performed by the Environmental Processes Branch (CN-E) of the Installations Division (CN) of the Construction Engineering Research Laboratory (CERL). Dr. Stephen W. Maloney was the CERL Project Manager; Clint M. Arnett and Giselle Rodriguez were the Principal Investigators. Ms. Debora Curtain is Chief, CN-E, and Dr. John T. Bandy is Chief, CN. Martin J. Savoie is Technical Director for the Installations business area. The Deputy Director of CERL is Dr. Kirankumar V. Topudurti, and the Director is Dr. Ilker R. Adiguzel.

CERL is an element of the U.S. Army Engineer Research and Development Center (ERDC), U.S. Army Corps of Engineers. The Commander and Executive Director of ERDC is COL Gary E. Johnston, and the Director of ERDC is Dr. James R. Houston.

Unit Conversion Factors

Multiply	By	To Obtain
degrees Fahrenheit	(F-32)/1.8	degrees Celsius
feet	0.3048	meters
gallons (U.S. liquid)	3.785412 E-03	cubic meters
pounds (mass)	0.45359237	kilograms

1 Introduction

Background

During Operation Desert Storm, the U.S. Army lost more armored vehicles in a single motor pool accident at Camp Doha than from hostile forces throughout the duration of the war. An ammunition carrier loaded with artillery shells caught fire resulting in a chain reaction of explosions throughout the motor pool, damaging and destroying over 100 vehicles (Cox and Perazzo 2004). Previous major incidents of propagating explosions have occurred on Navy ships, most notably the Forrestal in 1967 and Enterprise in 1969 (Darwin et al. 2005). To prevent such calamities from taking place in the future, the Department of Defense (DOD) has begun to develop Insensitive Munitions (IM) for use in future weapon systems. One result of the IM program has been the phasing out of traditional 2,4,6-trinitrotoluene (TNT) based explosives in favor of new chemical formulations. IM retain or surpass the performance of traditional explosives, but are characteristically more resistant to heat and shock detonation making them safer to manufacture, transport, and store.

Since the mid 1980s, Picatinny Arsenal has developed over 24 Picatinny Arsenal eXplosive (PAX) IM formulations. PAX-21 is one IM that is currently in production and has been test loaded at select Army ammunition plants. It is a cost effective melt-and-pour energetic designed to replace Composition-B, a conventional TNT-based explosive. PAX-21 contains a mixture of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), ammonium perchlorate, 2,4-dinitroanisole (DNAN), and *n*-methyl-4-nitroaniline (MNA). Although the exact composition of the energetic is proprietary, it is estimated that nearly half of the dry weight of PAX-21 is composed of DNAN and MNA. PAX-40 and PAX-41 are two additional IM that will likely be entering production in the near future. PAX-40 is an octol replacement containing a mixture of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), DNAN, and MNA. PAX-41 is a cyclotol replacement that contains a mixture of RDX, DNAN, and MNA.

As the DOD continues to transition from conventional munitions to IM substantial amounts of perchlorate, DNAN, and MNA will begin entering waste streams at production and load and pack facilities. RDX, TNB, DNAN, and MNA are all nitramine compounds (Figure 1), which

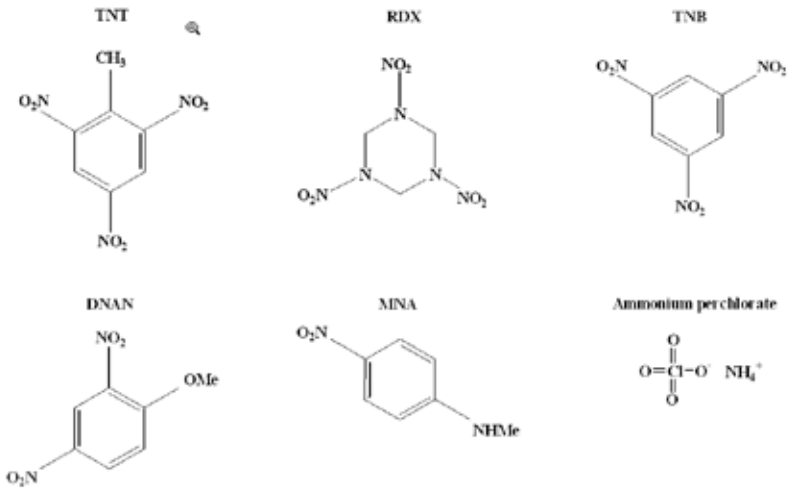

Figure 1. Structures of 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), 1,3,5-trinitrobenzene (TNB), 2,4-dinitroanisole (DNAN), *n*-methyl-4-nitroaniline (MNA), and ammonium perchlorate.

have a strong electron-withdrawing effect. This can result in enhanced reactivity towards nucleophiles and reducing agents (Schmitt et al. 2000). This electrophilic reactivity is of great concern due to its toxic effects in humans and wildlife (Talmage et al. 1999; Robidoux et al. 2000; USEPA 2006). Dodd and McDougal (2002) have demonstrated that DNAN is in fact more toxic than TNT, the explosive it is to replace. Due to the recalcitrant nature of nitrated compounds and their generalized toxicity, release into the environment is highly regulated. Thus, wastewater generated during production, load and pack, and demilitarization operations containing nitramines must be treated before discharge.

Historically, nitroaromatic and cyclic nitramine contaminant wastewater has been treated by granular activated carbon (GAC) absorption. Although GAC is very effective at removing munitions from production wastewater, the method generates large quantities of energetic-laden carbon, which is costly to dispose of (Concurrent Technologies Corp. 1995; Maloney et al. 2002). Biotransformation is considered a more economical treatment option that possesses the potential to transform explosive constituents to innocuous compounds. Many studies have demonstrated effective treatment of energetic contaminated wastewater using bioreactor technologies (VanderLoop et al. 1998; Maloney et al. 2002; U.S. Department of Defense 2003; Fuller et al. 2007; Atikovic et al. 2008).

Although the engineering aspects of bioreactors are fairly well understood, very little is known about the microbial communities present within. A better understanding of the effects of energetics on the population dynamics of bioreactor systems is a critical step towards enhancing degradative capabilities to meet future wastewater treatment needs. By identifying bacteria responsible for energetic degradation, conditions can be optimized to make treatment processes more efficient. To date, only two studies involving microbial diversity within nitramine treating reactors have been published. Adrian et al. (2001) characterized the microbial communities of Anaerobic Fluidized Bed Reactors (AFBR) treating TNT, and Kimura et al. (2003) characterized the microbial community in a 2,4-dinitrophenol (2,4-DNP) digesting bioreactor.

Ammonium perchlorate has been traditionally used in solid rocket fuels by the DOD, but is currently being added to many IM formulations. Perchlorate (ClO_4^-) is a highly soluble anion, which is relatively non-reactive under normal environmental conditions. The compound consists of a chlorine atom surrounded by four oxygen atoms (Figure 1). The halogenated inorganic absorbs poorly to humic and mineral surfaces, and is nonvolatile and chemically stable, which facilitates its transport through the environment. It is known to inhibit iodide transport in mammals by binding sodium-iodide symporter causing thyroid gland disruption (Stanbury and Wyngaarden 1952; Wolff 1998; Urbansky 2000). Due to the anions' toxicity the U.S. Environmental Protection Agency (USEPA) has set a recommended action level of 1 ppm in drinking water (Coates and Achenbach 2004). Therefore, industrial wastewater must be treated before discharge.

Because perchlorate is resistant to abiotic reduction, bioremediation has been targeted as a potential transformation technology. The compounds high reduction potential (ClO_4^-/Cl^- $E°$ = 1.287 V) makes it well suited as an electron acceptor for bacterial growth (Coates et al. 2000). Biological reduction of perchlorate laden industrial wastewater has been demonstrated for rocket demilitarization operations (ESTCP 2000), and is a feasible means of treating large quantities of contaminated groundwater (Hatzinger 2005). Additionally, biodegradation of perchlorate has been demonstrated in bioreactors commingled with high explosives (Fuller et al. 2007; Maloney et al. 2008). Several bacterial isolates have been shown to degrade perchlorate (Coates and Achenbach 2004). However, as with reactor systems treating nitrated compounds, characterization of bioreactor microbial communities are very limited.

Krauter et al. (2005) characterized wetland bioreactor treating perchlorate and nitrate by Denaturing Gradient Gel Electrophoresis (DGGE) analysis and found the dominant organisms within the system were *Pseudomonas*, *Acinetobacter*, *Halomonas*, and *Nitrospira* species. Zhang et al. (2005) characterized biofilms from a pilot-scale perchlorate-reducing bioreactor by 16S-23S ribosomal intrinsic spacer analysis and found the microbial community to be enriched with *Dechloromonas* species even though the reactor was inoculated with a perchlorate respiring *Dechlorosoma* species.

Objectives

The objectives of this study were to characterize the microbial diversity of and to identify principal groups of microorganisms within five bioreactors treating high explosives and IM.

Approach

The characterization of microbial communities by culture-based techniques results in biases because the vast majority of bacteria cannot be grown on defined media for identification purposes (Nocker et al. 2007). Due to inefficiencies of conventional characterization methods, a direct cloning and sequencing methodology was employed where 16S rRNA genes were used to characterize the microbiota within each reactor. Briefly, community genomic DNA was extracted from homogenized reactor samples, and a 900 bp segment of the 16S rRNA gene was amplified by PCR using select universal primers. The PCR amplicons were ligated into a plasmid cloning vector and transformed into competent bacterial cells, allowing self-replication of the inserted DNA fragments. Plasmids from recombinant clones were extracted, purified, and sequenced. To phylogenetically classify each clone, the base pair sequence was compared to known sequences in a ribosomal data base. Dominant organisms within each bioreactor were then identified.

Mode of technology transfer

The results of the DNAN and MNA AFBR study are to be published in peer reviewed journals. These data will also direct future laboratory studies with GLBR, which will eventually lead to design and operational procedures for full-scale bioreactors used for the treatment of IM contaminated wastewater. This report will be made accessible through the World Wide Web (WWW) at URL: http://www.cecer.army.mil

2 Materials and Methods

Bioreactor operation and sampling

AFBR treating DNAN and MNA

AFBR were designed and operated as described by Atikovic et al. (2008) (Figure 2). Briefly, three separate 9.1L anaerobic reactors were developed to evaluate the effectiveness of biological IM treatment. Bioreactor 1 (AFBR #1) treated DNAN, bioreactor 2 (AFBR #2) treated MNA, and bioreactor 3 (AFBR #3) treated a combination of both DNAN and MNA simultaneously. The bioreactor feed consisted of a mixture of a nutrient solution, a buffer solution, and a contaminant feed stock containing 600 ppm ethanol as an electron donor. The bioreactors were operated at 36 °C and pH was maintained at 7. The total aqueous flow rate was approximately 6L day^{-1} consisting of 0.5L nutrient stock, 0.5L buffer solution and 5L contaminant feed stock. The empty-bed Hydraulic Retention Time (HRT) was approximately 36 hours. The reactor was seeded with 50 ml of anaerobic digester sludge obtained from a pilot-scale system being operated at the Environmental Engineering and Science Laboratory of the University of Cincinnati. The reactors were allowed to stabilize and fluidization was optimized before the addition of IM. After a 200-day period 21 ppm DNAN and 4 ppm MNA were introduced in the feeds and allowed to acclimate for 30 days. The concentrations were then increased to 42 ppm DNAN and 8 ppm MNA. Throughout the treatment period effluent concentrations were monitored and DNAN and MNA remained below the high pressure liquid chromatography (HPLC) detection limits of 0.07 ppm and 0.1 ppm respectively.

Three ml homogenized mixed-liquor samples containing both liquid and sand were taken at day 200 before the addition of IM and after 60 days of IM treatment. Samples were immediately frozen until time of DNA extraction.

GLBR bioreactor treating perchlorate

GAC was used as the support for biological growth and hydrogen was supplied as a source of reducing equivalents. A 15 x 26 cm reactor was constructed of acrylic and stainless steel having a total bed volume of 5.0L and contained 287 g of Calgon Carbon Co. F-400 GAC (Pittsburgh, PA) as a support medium for biofilm formation (Figure 3).

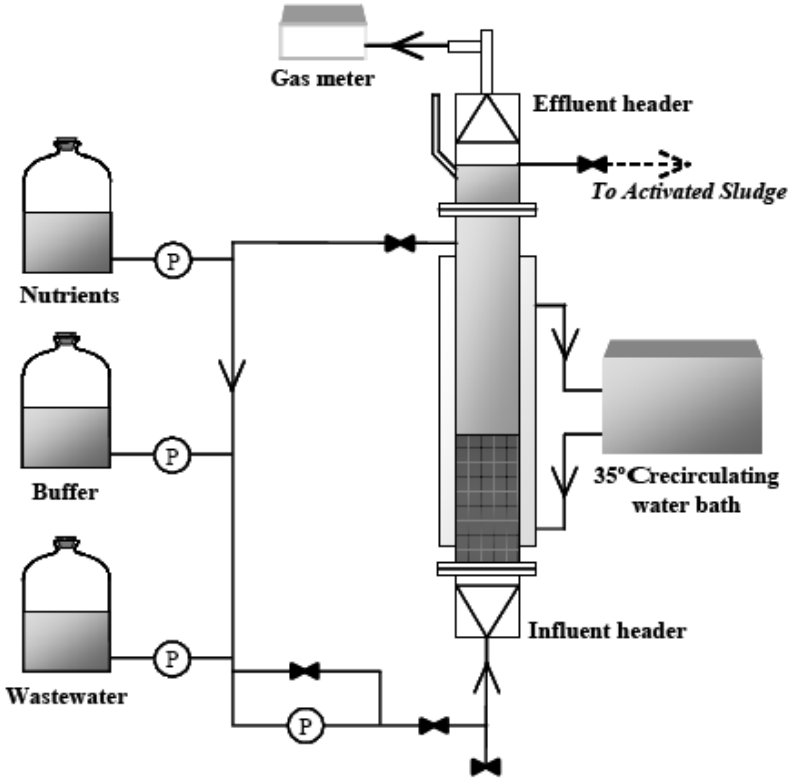

Figure 2. Schematic of anaerobic fluidized bed bioreactors treating 2,4-dinitroanisole (DNAN) and *n*-methyl-4-nitroaniline (MNA).

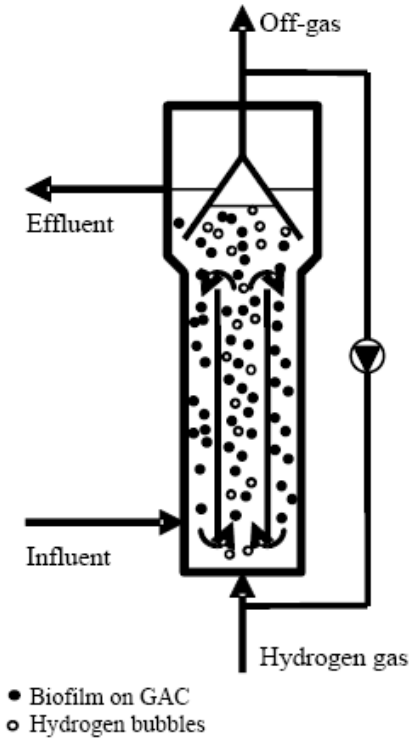

Figure 3. Schematic of a hydrogen-enhanced, gas lift, bioreactor (GLBR) treating ammonium perchlorate.

The reactor was operated using a synthetic wastewater as described by Nerenberg et al. (2005) and seeded with biomass collected from an anaerobic digester at the Champaign-Urbana Wastewater Treatment Plant (Urbana, IL). Hydrogen (95 percent H_2 : 5 percent CO_2) flow was 110 ml min^{-1}, which was collected from the headspace and recirculated at a rate of 3.0-4.5L min^{-1} through a ceramic diffuser at the base of the reactor. The introduction of hydrogen gas into a tube in the center of the reactor created a continuous recirculating flow pattern. Operational temperature was maintained at 30 °C and pH was maintained between 7.3 and 8.0 by adjusted by carbon dioxide concentration. GAC was periodically added to the system to replenish GAC loss due to washout and sampling. Perchlorate was introduced into the influent at a concentration of 100 ppm and HRT was approximately 3 hr. During treatment the reactor effluent was found to be below HPLC detection levels of less than 5 ppb perchlorate.

Ten ml homogenized mixed-liquor samples were taken after 180 days of perchlorate treatment and compared to the raw sludge collected from the wastewater treatment plant. All samples were immediately frozen until time of DNA extraction.

GAC-FBR treating RDX, TNB, and TNT

A demonstration scale GAC-FBR was installed at the McAlester Army Ammunition Plant (MCAAP) under the Environmental Security Technology Certification Program (ESTCP) for the treatment of RDX and TNT contaminated wastewater. The system (Figure 4) was constructed and operated as described by Maloney et al. (2002) and ESTCP (2003).

Briefly, the system consists of a cylinder 1.4 m in diameter and 6.7 m tall (total volume 9,900L) and a 0.9 m diameter by 1.5 m tall separator tank, which collects biomass and GAC washout and recirculates it back into the reactor. Modifications to the original ESTCP design included elevating the separator tank to eliminate hydraulic resistance and substituting the nutrient media with Opticor Range Minerals (Archer Daniels Midland, Decatur, IL). The reactor operated at a flow rate of 4 to 23L day^{-1} and had a HRT of 7 to 44 hours. Reactor influent concentrations were 57 ppm TNT, 3 ppm TNB and 540 ppb RDX. TNT, TNB, and RDX were not observed in the reactor effluent at HPLC detection limits of 2 ppb, 3 ppb, and 5 ppb respectively. The demonstration plant bioreactor was operating for more than 2 years on a continuous basis before sampling.

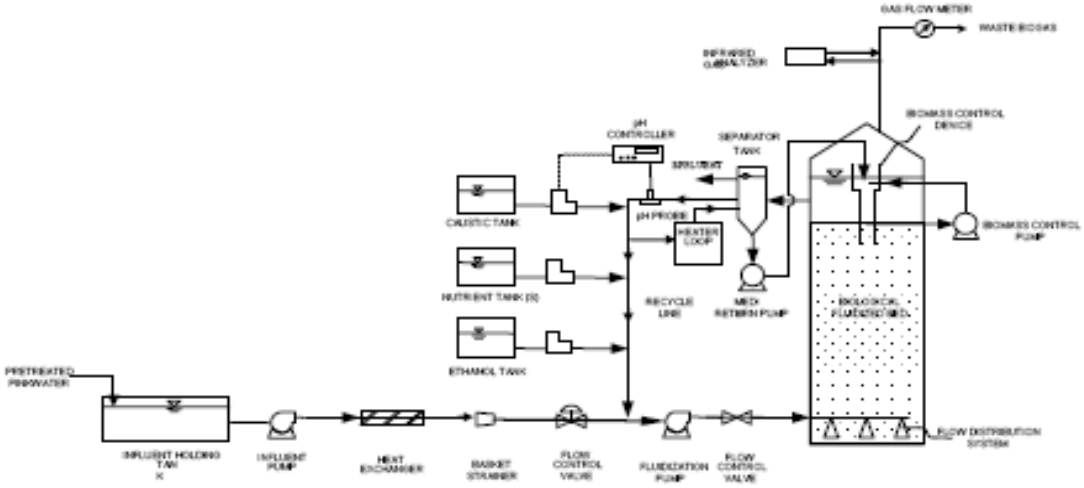

Figure 4. Schematic of the McAlester Army Ammunition Plant (MCAAP) granulated activated carbon, fluidized bed, bioreactor (GAC-FBR) treating 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 1,3,5-trinitrobenzene (TNB).

A 50 ml homogenized mixed-liquor sample containing both liquid and GAC was taken from the top of the reactor during explosive wastewater treatment. The sample was immediately frozen until time of DNA extraction.

DNA extraction and 16S rDNA PCR amplification

Total genomic DNA was extracted from 1 ml of each mixed-liquor sample using an UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) per the manufacturer's maximum yield protocol. The 16S rRNA gene was PCR amplified with 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 926R (5'-CCGTCAATTCCTTTRAGTTT-3') universal bacterial primers using Platinum *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA) and a MJ Research PTC 200 thermal cycler (Bio-Rad Laboratories, Hercules, CA). Final reaction volumes were 50 µl having the following concentrations:

- Platinum *Taq* PCR buffer, 1x
- $MgCl_2$, 1.5 mM
- dNTPs (1:1:1:1), 0.1 mM
- universal primer, 0.2 µM
- BSA, 5 µg
- Platinum *Taq* DNA Polymerase, 0.1 U
- genomic DNA, 20 ng.

Thermal cycler parameters were 95 °C for 3 minutes then 20 cycles of 45 sec at 94 °C, 1 minutes at 59 °C, 2 minutes at 72 °C followed by a final ex-

tension at 72°C for 10 minutes. Three PRC reactions were performed for each sample and pooled before cloning. Additionally, reactions were kept to 20 cycles to minimize PCR biases and to better represent the true 16S rRNA gene distribution (Kanagawa 2003). These PCR conditions resulted in roughly a 900 bp amplicon. All amplicons were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA) as directed by the manufacturer. Both DNA extractions and purified 16S rRNA gene PCR amplifications were quantified using an Invitrogen Quan-iT DNA assay kit per the manufacture's instructions. Purity was confirmed by gel electrophoresis using a SYBR Safe (Invitrogen) 1 percent agarose gel ran at 70 V for 45 minutes. Gels were visualized under UV light and products were sized using a 100 bp DNA ladder (Promega, Madison, WI).

Construction of bacterial 16S rDNA clone libraries

The purified PCR amplicons were cloned using an Invitrogen TOPO TA Cloning Kit per the manufacturer's instructions and transformed into chemically competent *Escherichia coli* cells. Ten microliters of each transformation reaction was plated onto LB broth (Becton Dickinson, Sparks, MD) containing 100 μg ml^{-1} spectinomycin and incubated overnight at 37 °C. One hundred random, well isolated, spectinomycin resistant colonies were picked from the plates and grown overnight at 37 °C in liquid LB containing 100 μg ml^{-1} spectinomycin. Random clones were screened for recombination by PCR amplification as described previously using M13 forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers. Products were sized on a SYBR Safe 1 percent agarose gel as described above.

Clone 16S rDNA sequencing

Overnight bacterial cultures were transferred into 0.45 ml 96 well culture plates containing LB and 100 μg ml^{-1} spectinomycin. After 24 hours growth at 37 °C, plasmid DNA was extracted from the cultures using heat lysis, where the cells were washed in phosphate buffer and then heated to 95 °C. Lysed cells were spun down and the supernatant, containing the plasmid DNA, was transferred to a new 96-well plate. Sequencing reactions were set up as follows:

- 4μl of 12.5 percent glycerol
- 2μl of 5x Applied Biosystems (ABI) Sequencing Buffer (Applied Biosystems, Forest City, CA)
- 1 μl of 1 μM M13 forward primer
- 0.75 μl of water

- 0.25 µl of ABI BigDye Terminator v3.1
- 2 µl of plasmid template per sample.

Thermal cycling was performed at 96°C for 5 minutes followed by 35 cycles of 96°C for 15 sec, 45°C for 5 sec and 60°C for 4 minutes. Reaction products were precipitated with 70 µl of 0.2 mM MgSO4 in 70 percent ethanol for 15 minutes then spun at 3600 rpm for 15 minutes. Plates were inverted and spun at 1000 rpm for 1 minute to remove precipitation solution. The precipitated pellets were dried in the Savant SpeedVac (Thermo Fisher Scientific, Waltham, MN) for 10 minutes to remove residual ethanol. Samples were re-suspended in 10µl ABI HiDi Formamide and denatured at 95 °C for 5 minutes, then loaded onto the ABI 3730xl equipped with a 50 cm 96-capillary array. Samples were run using a modified version of the default LongSeq50_POP7 run module, where injection time was increased to 25 seconds and run time decreased to 84 minutes. Samples were analyzed with ABI Analysis v5.2 software.

Phylogenetic analysis

Sequencing reads were manually edited with Sequencher v4.2 computational sequencing software (Gene Codes Corporation, Ann Arbor, MI). Approximately 550 clones were successfully sequenced having an average length of roughly 700 bp. All sequences were examined for chimera using CHIMERA_CHECK v2.7 chimera detection program* (Cole et al. 2003) and suspect sequences were removed from the data set. Sequences were aligned with Sequencher v4.2 resulting in the identification of 114 unique clone species. The Ribosomal Database Project II (RDP) Release 9.51 *Classifier* program† was used to assign each unique clone to principal hierarchical taxa based on a naïve Bayesian rRNA classification (Wang et al. 2007). Both type and non-type strains of uncultured and isolated organisms were searched. Closest GenBank nucleotide sequence matches were compiled using the Basic Local Alignment Search Tool (BLAST) algorithm‡ based on maximum BLAST scores (Zhang et al. 2000).

* http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU

† http://rdp.cme.msu.edu/classifier/classifier.jsp

‡ http://www.ncbi.nlm.nih.gov/blast/Blast.cgi

3 Results

AFBR treating DNAN and MNA clone distribution

Cloning and sequencing the 16S rRNA genes from the three AFBRs identified the presence of bacteria belonging to six unique phyla (Table 1). Before the addition of DNAN to AFBR #1, the majority of the microbial population consisted of member belonging to the phylum *Proteobacteria* (76 percent). The same trend was also observed in AFBR #2 and AFBR #3 where 83 and 79 percent of the clone distributions also belonged to the phylum *Proteobacteria*, respectively.

However, during the treatment of DNAN and MNA, the microbial population dynamics shifted dramatically. After the 60-day treatment period, the *Proteobacteria* populations dropped to less than 6, 12, and 16 percent of the total clone distribution in the reactors treating DNAN, MNA, and DNAN/MNA, correspondingly. Bacteria belonging to the phylum *Chloroflexi* became the dominant species in each reactor system. The *Chloroflexi* population increased from 1 percent of the population to 42 percent of the population in AFBR #1, 10 percent to 39 percent in AFBR #2, and 11 percent to 30 percent in AFBR #3.

Table 1. Bacterial community phylogenetic distribution in 3 anaerobic fluidized bed bioreactors (AFBR) treating 2,4-dinitroanisole (DNAN) and *n*-methyl-4-nitroaniline (MNA) based on 16S rDNA clone distribution.

	AFBR #1				AFBR #2				AFBR #3			
	DNAN (0 ppm)		DNAN (42 ppm)		MNA (0 ppm)		MNA (8 ppm)		DNAN (0 ppm) MNA (0 ppm)		DNAN (42 ppm) MNA (8 ppm)	
Phylum[a]	Sp.[b]	Clone (%)[c]	Sp.	Clone (%)[d]	Sp.	Clone (%)[e]	Sp.	Clone (%)[f]	Sp.	Clone (%)[g]	Sp.	Clone (%)[h]
Bacteroidetes	2	3	1	3	0	0	2	3	2	2	4	14
Chloroflexi	1	1	2	42	2	10	1	39	1	11	2	30
Firmicutes	0	0	5	9	0	0	1	9	0	0	2	4
Nitrospira	1	1	1	2	0	0	1	1	1	1	1	4
Proteobacteria	5	76	3	6	1	83	3	12	4	79	3	16
Spirochaetes	0	0	1	2	0	0	0	0	1	2	0	0
unclassified bacteria[i]	3	19	6	36	4	7	7	36	2	5	8	32

[a] Determined with the RDP Classifier program using naïve Bayesian rRNA classifier (Wang et al. 2007).
[b] Number of unique species within the phylum.
[c] Percent of clones out of a total of 79 before the addition of DNAN.
[d] Percent of clones out of a total of 90 after 60 days of DNAN treatment.
[e] Percent of clones out of a total of 91 before the addition of MNA.
[f] Percent of clones out of a total of 89 after 60 days of MNA treatment.
[g] Percent of clones out of a total of 90 before the addition of DNAN/MNA.
[h] Percent of clones out of a total of 90 after 60 days of DNAN/MNA treatment.
[i] Bacterial sequences with no close matches within the RDP.

In addition, a 12 percent increase in the *Bacteroidetes* population was observed in AFBR #3. A population of *Firmicutes* increased 8 percent in the reactor treating MNA and 4 percent in the reactor treating DNAN/MNA. A substantial increase in unidentified bacteria was also observed in all three reactor systems. All other species representing the remaining phyla increased or degreased by less than or equal to 3 percent of the total population.

Table 2 lists the preliminary clone classifications within AFBR #1. Before the addition of DNAN, the reactor was composed of twelve unique bacterial species belonging to four distinct phyla: *Bacteroidetes*, *Chloroflexi*, Nitrospira, and *Proteobacteria*. The majority of the clone distribution (70 percent) was made up of a single unclassified *Desulfuromonales* species (clone UC-19). A GenBank BLAST search revealed the clone was most closely related to uncultured bacterium clone AME E37, having a 98 percent sequence similarity. The second most abundant clone (UC-15), making up 9 percent of the clone distribution, was an unclassified bacterium. The third most abundant clone (UC-13) was yet another unclassified bacterium, which made up 6 percent of the population. All other clones made up less than 4 percent of the total clone distribution.

Addition of DNAN to the reactor had a significant effect on the dynamics of the microbial population (Table 2). The number of unique bacteria increased from 12 to 19 unique species. Interestingly, the total *Proteobacteria* population dropped from 76 to 6 percent of the clone distribution over 60 days of treatment. Within this same period the unclassified *Desulfuromonales* population decreased to 3 percent of the population. Three bacteria belonging to the phylum *Chloroflexi* were identified in the reactor, two unclassified Caldilineacea species (clones UC-7 and UC-36) and one *Levilinea sp.* (clone UC-14).

The Caldilineacea clones had 95 percent sequence identities to uncultured bacterial clones SHD-157 and ZZ14AC19, respectively. The *Levilinea sp.* was most closely related to uncultured bacterial clone SHD-238 (96 percent). Clone UC-7 was not detected before the addition of DNAN and only made up 1 percent of the population after 60 days of incubation. Clone UC-36 composed 1 percent of the population before the addition of DNAN, but was not detected after 60 days of treatment. The *Levilinea sp.* was undetected before treatment, yet became the dominant organism in the presences of DNAN at greater than 41 percent of the population.

Table 2. Ribosomal DNA clone distribution in an anaerobic fluidized bed bioreactor (AFBR #1) treating 2,4-dinitroanisole (DNAN).

Clone	Preliminary classification[a]	GenBank match[b]	DNAN (0 ppm)		DNAN (42 ppm)	
			Freq[c]	%	Freq[d]	%
UC-1	Bacteria/unclassified	98%, uncultured anaerobic clone bacterium B-1AC, AY953152	3	3.8	0	0.0
UC-2	*Clostridia*/unclassified	96%, uncultured bacterium clone LS4-180, AB234270	0	0.0	1	1.1
UC-3	Incertae Sedis XV/ unclassified	98%, uncultured bacterium clone N42, AB19586	0	0.0	1	1.1
UC-4	Ruminococcaceae/ unclassified	96%, uncultured bacterium clone HDBW-WB60, AB237723	0	0.0	1	1.1
UC-5	*Veillonellaceae*/ unclassified	95%, uncultured bacterium clone AKIW991, DQ129277	0	0.0	1	1.1
UC-6	Bacteria/unclassified	99%, uncultured bacterium clone TANB22, AY667254	0	0.0	1	1.1
UC-7	Caldilineacea/unclassified	95%, uncultured bacterium clone SHD-157, AJ306795	0	0.0	1	1.1
UC-13	Bacteria/unclassified	99%, uncultured bacterium clone SHA-41, AJ306787	5	6.3	0	0.0
UC-14	Levilinea	96%, uncultured bacterium clone SHD-238, AJ278171	0	0.0	37	41.1
UC-15	Bacteria/unclassified	91%, uncultured bacterium clone Pav-079, DQ642388	7	8.9	0	0.0
UC-16	Bacteroidales/unclassified	99%, uncultured bacterium clone S25, AB195881	1	1.3	3	3.3
UC-18	Magnetobacterium	100%, N02, AB195896	1	1.3	2	2.2
UC-19	Desulfuromonales/ unclassified	98%, uncultured bacterium clone AME E37, DQ191707	55	69.6	3	3.3
UC-20	Bacteria/unclassified	92%, ORSFC1_c1, EF393361	0	0.0	1	1.1
UC-21	Delta*Proteobacteria*/ unclassified	98%, uncultured Syntrophorhabdaceae bacterium SHA-22, AJ249102	0	0.0	1	1.1
UC-22	Smithella	88%, uncultured *Syntrophus sp.*; KB-1, AY780562	1	1.3	1	1.1
UC-23	Delta*Proteobacteria*/ unclassified	94%, uncultured bacterium clone Er-LLAYS-48, EU542511	1	1.3	0	0.0
UC-24	Bacteria/unclassified	85%, uncultured bacterium clone BE325FW032701CTS_hole1-17, DQ088767	0	0.0	1	1.1
UC-25	*Bacteroidetes*/unclassified	92%, uncultured bacterium clone LWS-T4819, EU546335	1	1.3	0	0.0
UC-26	Dechloromonas	100%, *Dechloromonas sp.* HZ, AF479766	2	2.5	0	0.0
UC-27	Bacteria/unclassified	99%, uncultured bacterium clone N16, AB195913	0	0.0	1	1.1
UC-28	*Veillonellaceae*/ unclassified	95%, uncultured bacterium clone NGA32, EF613951	0	0.0	4	4.4
UC-29	Bacteria/unclassified	91%, uncultured bacterium clone 30f03, EF515591	0	0.0	1	1.1
UC-30	Delta*Proteobacteria*/ unclassified	97%, uncultured bacterium clone AV9-8, AM181814	1	1.3	0	0.0
UC-32	*Spirochaetaceae*/ unclassified	98%, Spirochaetes bacterium SA-10, AY695841	0	0.0	2	2.2
UC-34	Bacteria/unclassified	99%, *Chlorobi* bacterium clone Ev219h1bfT3b37, EF446822	0	0.0	27	30.0
UC-36	Caldilineacea/unclassified	95%, uncultured bacterium clone ZZ14AC19, AY214200	1	1.3	0	0.0

[a] Determined with RDP Classifier program using naïve Bayesian rRNA classifier (Wang et al. 2007).
[b] GenBank sequences most closely related to the 16S rDNA clone sequences. Accession numbers and sequence identities based on maximum BLAST scores are listed.
[c] Frequency of clones out of a total of 79 before the addition of DNAN.
[d] Frequency of clones out of a total of 90 after 60 days of DNAN treatment.

Clone UC-34 (unclassified bacteria), which was not detected in the reactor before the addition of DNAN, became the second most abundant organism after the 60 days treatment period at 30 percent of the total clone distribution. The clone had a 99 percent sequence similarity to an uncultured *Chlorobi* bacterium clone Ev219h1bfT3b37. A *Veillonellaceae* species (clone UC-28) was also detected at 4 percent of the distribution. All clones remaining comprised less than 4 percent of the total clone distribution.

Table 3 lists the 16S rDNA clone distribution within AFBR #2. Before the addition of MNA, the reactor was composed of a total of seven unique bacterial species. As with AFBR #1, the microbial population was dominated by clone UC-19 (unclassified *Desulfuromonales*) at 84 percent of the clone distribution.

Table 3. Ribosomal DNA clone distribution in an anaerobic fluidized bed bioreactor (AFBR #2) treating *n*-methyl-4-nitroaniline (MNA).

Clone	Preliminary classification[a]	GenBank match[b]	MNA (0 ppm)		MNA (15 ppm)	
			Freq[c]	%	Freq[d]	%
UC-8	Caldilineacea/unclassified	95%, uncultured bacterium, BEU-45, EF053112	1	1.1	0	0.0
UC-9	Bacteria/unclassified	100%, uncultured bacterium clone SmB103; AB372572	0	0.0	5	5.6
UC-13	Bacteria/unclassified	99%, uncultured bacterium clone SHA-41, AJ306787	3	3.3	7	7.9
UC-14	Levilinea	96%, uncultured bacterium clone SHD-238, AJ278171	8	8.8	32	36.0
UC-15	Bacteria/unclassified	91%, uncultured bacterium clone Pav-079, DQ642388	1	1.1	4	4.5
UC-16	Bacteroidales/unclassified	99%, uncultured bacterium clone S25, AB195881	0	0.0	2	2.2
UC-17	Bacteria/unclassified	89%, uncultured bacterium clone SHA-83, AJ306807	1	1.1	12	13.5
UC-18	Magnetobacterium	100%, N02, AB195896	0	0.0	1	1.1
UC-19	Desulfuromonales/ unclassified	98%, uncultured bacterium clone AME E37, DQ191707	76	83.5	8	8.9
UC-20	Bacteria/unclassified	92%, ORSFC1_c1, EF393361	1	1.1	1	1.1
UC-21	Delta*Proteobacteria*/unclassified	98%, uncultured Syntrophorhabdaceae bacterium SHA-22, AJ249102	0	0.0	2	2.2
UC-23	Delta*Proteobacteria*/unclassified	94%, uncultured bacterium clone Er-LLAYS-48, EU542511	0	0.0	1	1.1
UC-24	Bacteria/unclassified	85%, uncultured bacterium clone BE325FW032701CTS_hole1-17, DQ088767	0	0.0	4	4.5
UC-25	*Bacteroidetes*/unclassified	92%, uncultured bacterium clone LWS-T4819, EU546335	0	0.0	1	1.1
UC-29	Bacteria/unclassified	91%, uncultured bacterium clone 30f03, EF515591	0	0.0	1	1.1
UC-31	Fusibacter	99%, uncultured bacterium clone LO3_CL-100643_OTU-9, EU808971	0	0.0	8	8.9

[a] Determined with RDP Classifier program using naïve Bayesian rRNA classifier (Wang et al. 2007).
[b] GenBank sequences most closely related to the 16S rDNA clone sequences. Accession numbers and sequence identities based on maximum BLAST scores are listed.
[c] Frequency of clones out of a total of 91 before the addition of MNA.
[d] Frequency of clones out of a total of 89 after 60 days MNA treatment.

The second most abundant clone, which made up 9 percent of the clone distribution, was clone UC-14 (*Levilinea sp.*). One additional *Chloroflexi* species (clone UC-8) was also identified, which comprised 1 percent of the population. The remaining clones were four species of unclassified bacteria (UC-13, UC-15, UC-17 and UC-20) that collectively made up 7 percent of the total clone distribution.

The addition of MNA to the reactor system increased the total number of bacteria to 15 unique species (Table 3). A similar shift in population dynamics was observed in the MNA amended reactor as with the DNAN amended reactor. The *Desulfuromonales* population decreased to less than 9 percent of the population and the *Levilinea* population increased to 36 percent of the population over the 60-day treatment period. One *Firmicutes* species (clone UC-31) was identified, which made up 9 percent of the population. Seven species of unclassified bacteria (UC-13, UC-15, UC-17, UC-20, UC-24, and UC-29), which collectively made up 38 percent of the total clone distribution, were identified. However, clone UC-34 was not detected in the presence of MNA. All remaining clones comprised less than 2 percent of the total clone distribution.

Table 4 lists the bacterial phylogenetic distribution within AFBR #3. Before the addition of DNAN and MNA, the reactor was comprised of a total of 11 unique bacterial species. Seventy nine percent of the population was made up of bacteria from the phylum *Proteobacteria* and 11 percent from the phylum *Chloroflexi*. The addition of IM to the system increased the total number of unique species to 20, and a similar shift in population dynamics was observed, as was seen in AFBRs #1 and #2. Clone UC-19 (uncultured *Desulfuromonales*) initially dominated the microbial population at 74 percent of the distribution. However, it decreased to 12 percent of the distribution after the 60-day treatment period. UC-14 (*Levilinea sp.*) was the second most abundant clone within the system making up 11 percent of the clone distribution before the addition of IM. After the treatment period, the *Levilinea* population increased to nearly 28 percent of the clone distribution. Eight species of unclassified bacteria (UC-12, UC-13, UC-15, UC-17, UC-20, UC-24, UC-37, and UC-34) making up 32 percent of the total clone distribution were also identified in the bioreactor. Clone UC-34 made up only 1 percent of the total distribution in the presence of IM.

Table 4. Ribosomal DNA clone distribution in an anaerobic fluidized bed bioreactor (AFBR #3) treating 2,4-dinitroanisole (DNAN) and *n*-methyl-4-nitroaniline (MNA).

Clone	Preliminary Classification[a]	GenBank match[b]	DNAN (0 ppm) & MNA (0 ppm)		DNAN (42 ppm) & MNA (8 ppm)	
			Freq[c]	%	Freq[d]	%
UC-10	Bacteria/unclassified	92%, uncultured bacterium clone, JML_C04; AM746236	1	1.1	0	0.0
UC-11	Bacteroidales/unclassified	99%, uncultured bacterium clone MR41, DQ661708	0	0.0	1	1.1
UC-12	Bacteria/unclassified	99%, uncultured *Firmicutes* bacterium, Ctrl2-2D, EU522662	0	0.0	1	1.1
UC-13	Bacteria/unclassified	99%, uncultured bacterium clone SHA-41, AJ306787	0	0.0	6	6.7
UC-14	Levilinea	96%, uncultured bacterium clone SHD-238, AJ278171	10	11.1	25	27.8
UC-15	Bacteria/unclassified	91%, uncultured bacterium clone Pav-079, DQ642388	3	3.3	9	10.0
UC-16	Bacteroidales/unclassified	99%, uncultured bacterium clone S25, AB195881	1	1.1	8	8.9
UC-17	Bacteria/unclassified	89%, uncultured bacterium clone SHA-83, AJ306807	0	0.0	5	5.6
UC-18	Magnetobacterium	100%, N02, AB195896	1	1.1	4	4.4
UC-19	Desulfuromonales/unclassified	98%, uncultured bacterium clone AME E37, DQ191707	67	74.4	11	12.2
UC-20	Bacteria/unclassified	92%, ORSFC1_c1, EF393361	0	0.0	1	1.1
UC-21	Delta*Proteobacteria*/unclassified	98%, uncultured Syntrophorhabdaceae bacterium SHA-22, AJ249102	0	0.0	1	1.1
UC-22	Smithella	88%, uncultured *Syntrophus sp.*; KB-1, AY780562	2	2.2	0	0.0
UC-23	Delta*Proteobacteria*/unclassified	94%, uncultured bacterium clone Er-LLAYS-48, EU542511	1	1.1	0	0.0
UC-24	Bacteria/unclassified	85%, uncultured bacterium clone BE325FW032701CTS_hole1-17, DQ088767	0	0.0	5	5.6
UC-25	*Bacteroidetes*/Unclassified	92%, uncultured bacterium clone LWS-T4819, EU546335	1	1.1	1	1.1
UC-26	Dechloromonas	100%, *Dechloromonas sp.* HZ, AF479766	0	0.0	2	2.2
UC-27	Bacteria/unclassified	99%, uncultured bacterium clone N16, AB195913	0	0.0	1	1.1
UC-30	Delta*Proteobacteria*/unclassified	97%, uncultured bacterium clone AV9-8, AM181814	1	1.1	0	0.0
UC-31	Fusibacter	99%, uncultured bacterium clone LO3_CL-100643_OTU-9, EU808971	0	0.0	2	2.2
UC-32	Spirochaetaceae/unclassified	98%, Spirochaetes bacterium SA-10, AY695841	2	2.2	0	0.0
UC-33	Bacteroidales/unclassified	96%, uncultured bacterium clone HDBW-WB40; AB237703	0	0	3	3.3
UC-34	Bacteria/unclassified	99%, *Chlorobi* bacterium clone Ev219h1bfT3b37, EF446822	0	0.0	1	1.1
UC-35	Clostridium	99%, uncultured bacterium clone HDBW-WB51, AB237714	0	0.0	2	2.2
UC-36	Caldilineacea/unclassified	95%, uncultured bacterium clone ZZ14AC19, AY214200	0	0.0	1	1.1

[a] Determined with RDP Classifier program using naïve Bayesian rRNA classifier (Wang et al. 2007).
[b] GenBank sequences most closely related to the 16S rDNA clone sequences. Accession numbers and sequence identities based on maximum BLAST scores are listed.
[c] Number of clones out of a total of 90 before the addition of DANA and MNA.
[d] Number of clones out of a total of 90 after 60 days DNAN/MNA treatment.

GLBR treating perchlorate clone distribution

Cloning and sequencing the 16S rRNA genes from the raw sewage used to inoculate the GLBR identified 28 unique clones belonging to six distinct phyla (Table 5). *Bacteroidetes* were found to be the most abundant bacteria within the inoculum at 28 percent, followed by *Firmicutes* at 18 percent, *Chloroflexi* at 11 percent, and *Proteobacteria* at 10 percent. Two species, one belonging to the phylum Planctomycetes and the other to phylum Spirochaetes were also identified, which combined made up approximately 3 percent of the total clone distribution. Roughly 30 percent of the total bacterial population consisted of unidentifiable bacteria. After 180 days of perchlorate treatment, the GLBR was found to be dominated by *Proteobacteria*, which collectively made up greater than 62 percent of the reactor population (Table 5). The number of unique species belonging to the phyla *Bacteroidetes* and *Firmicutes* remain nearly the same; however, the total populations decreased to approximately 8 percent each. Additionally, the number of unidentified bacteria also decreased to roughly 9 percent of the clone distribution.

Table 5. Bacterial community phylogenetic distribution in a hydrogen-enhanced, gas lift, bioreactor (GLBR) treating ammonium perchlorate.

	Raw Sewage		GLBR	
Phylum[a]	Sp.[b]	Clone (%)[c]	Sp.	Clone (%)[d]
Actinobacteria	0	0	2	3.5
Bacteroidetes	6	28.2	7	8.2
Chloroflexi	2	11.3	2	3.5
Firmicutes	7	18.3	5	8.2
Lentisphaerae	0	0	1	1.2
OP10	0	0	0	0
Planctomycetes	1	1.4	0	0
Proteobacteria	3	10.0	4	62.4
Spirochaetes	1	1.4	1	1.2
Thermotogae	0	0	1	1.2
Verracomicrobia	0	0	1	1.2
unclassified bacteria[e]	8	29.7	5	9.4

[a] Determined with the RDP Classifier program using naïve Bayesian rRNA classifier (Wang et al. 2007).
[b] Number of unique species within the phylum.
[c] Percent of clones belonging to the phylum out of a total of 71 successfully sequenced clones.
[d] Percent of clones belonging to the phylum out of a total of 85 successfully sequenced clones.
[e] Bacterial sequences with no close matches within the RDP.

Of the 28 clones that were sequenced from the raw sewage, an unclassified *Bacteroidetes* (clone UI-14) was found to have the greatest abundance at approximately 14 percent of the total clone distribution (Table 6). A GenBank search revealed the clone had a 90 percent sequence similarity to uncultured bacterial clone SR24. The second most abundant clone was an unidentified bacterium (clone UI-12) at roughly 10 percent of the clone population. Clone UI-15 (unclassified Bacteroidales) made up approximately 6 percent of the distribution. Clone UI-17 (unclassified Caldilineacea) made up 4 percent of the population and was identified as toluene-degrading methanogenic consortium bacterium Eub 4. Clone UI-19, identified as *Comamonas aquatica*, also consisted of 4 percent of the population. Species of *Levilinea* (clone UI-16) and *Syntrophomonas* (clone UI-24) were also identified in the inoculum at 7 and 9 percent of the distribution, respectively. The *Levilinea sp.* had a 90 percent sequence match to an uncultured *Chloroflexi* bacterium and the *Syntrophomonas sp.* had a 92 percent sequence match to *Syntrophomonas sapovorans* (T). Clones UI-10 and UI-13 (unclassified bacteria) comprised roughly 6 percent of the population each. All remaining clones comprised less than 3 percent of the total clone distribution.

Table 6. Ribosomal DNA clone distribution in raw sewage collected from a wastewater treatment plant.

Clone	Preliminary classification[a]	GenBank match[b]	Clone	
			Freq c	%
UI-1	*Clostridia*/unclassified	85%, uncultured bacterium; BSA1B-03; AB175357	1	1.4
UI-2	Spirochaetaceae/unclassified	91%, uncultured bacterium; MTSBac-A8; EU591632	1	1.4
UI-3	Bacteria/unclassified	88%, uncultured bacterium; FRC-A2_451; EF508024	1	1.4
UI-4	Incertae Sedis XV/unclassified	93%, uncultured bacterium; ATB-KM1365; DQ390307	1	1.4
UI-5	Erysipelotricha-ceae/unclassified	92%, uncultured bacterium; MR4; DQ661698	1	1.4
UI-6	Bacteria/unclassified	92%, uncultured bacterium; BS14; EU358689	1	1.4
UI-7	Bacteria/unclassified	94%, unidentified bacterium; K2-30-7; AY344400	1	1.4
UI-8	Sedimentibacter	84%, uncultured bacterium; BSA1B-05; AB175359	1	1.4
UI-9	Planctomyceta-ceae/unclassified	90%, uncultured bacterium; RB-2C12; FJ172792	1	1.4
UI-10	Bacteria/unclassified	94%, uncultured bacterium; ZZ-S4E7; EF613425	4	5.6
UI-11	Bacteria/unclassified	93%, uncultured candidate division OP8 bacterium; b3cf12f09.rrna.clone5; AJ937676	2	2.8
UI-12	Bacteria/unclassified	92%, uncultured bacterium; MBF16_34; AB290396	7	9.9
UI-13	Bacteria/unclassified	89%, uncultured anaerobic bacterium; B-1AC; AY953152	4	5.6
UI-14	*Bacteroidetes*/unclassified	90%, uncultured rumen bacterium; SR24; DQ394627	10	14.1
UI-15	Bacteroidales/unclassified	99%, uncultured bacterium; B103; EF029344	4	5.6
UI-16	Levilinea	90%, uncultured *Chloroflexi* bacterium; A79; EF029425	5	7.0
UI-17	Caldilineacea/unclassified	100%, toluene-degrading methanogenic consortium bacterium; Eub 4; AF423184	3	4.2
UI-18	*Firmicutes*/unclassified	89%, uncultured bacterium; ATB-KM1223; DQ390265	2	2.8
UI-19	Comamonas	100%, *Comamonas aquatica*; LMG 5937; AJ430346	3	4.2
UI-20	Bacteroidales/unclassified	90%, uncultured bacterium; Eb6; EF063616	2	2.8
UIS-21	Bacteroidales/unclassified	85%, uncultured bacterium; c5LKS10; AM086109	1	1.4
UI-22	Syntrophaceae/unclassified	92%, uncultured bacterium; MBF16_A; AB290384	2	2.8
UI-23	Bacteria/unclassified	94%, uncultured low G+C Gram-positive bacterium; ML635J-28; AF507893	1	1.4
UI-24	Syntrophomonas	92%, Syntrophomonas sapovorans (T); AF022249	6	8.5
UI-25	Ruminococcaceae/unclassified	87%, uncultured bacterium; 1103200830322; EU842787	1	1.4
UI-26	Bacteroidales/unclassified	90%, uncultured bacterium; TP4_C; AB330842	2	2.8
UI-27	Acidisphaera	91%, *Rhodovastum atsumiense*; G2-11; AB381935	2	2.8
UI-28	*Bacteroidetes*/unclassified	84%, uncultured bacterium; N1903_75; EU104331	1	1.4

[a] Determined with RDP Classifier program using naïve Bayesian rRNA classifier (Wang et al. 2007).
[b] GenBank sequences most closely related to the 16S rDNA clone sequences. Accession numbers and sequence identities based on maximum BLAST scores are listed.
[c] Frequency of clones out of a total of 71.

Eighty five clones from the GLBR were successfully sequenced, of which 31 were found to be unique (Table 7). Only two clones identified in the raw sewage (UI-10 and UI-15) were identified in the GLBR after the 180-day treatment period. The hydrogen enhanced reactor was dominated by *Dechloromonas sp.* JM (clone UI-46) at greater than 55 percent of the total clone distribution. The second most abundant clone was UI-49 (unclassified bacteria) at 5 percent of the distribution. The third most abundant bacteria were species of *Ancalomicrobium* (clone UI-47) and *Sedimentibacter* (clone UI-55) making up approximately 4 percent of the population each. Clone UI-47 was found to be most closely related to *Rhizobiales* bacterium TP249 (98 percent) and clone UI-55 to uncultured bacterium clone ATB-KM1254 (96 percent). All remaining clones from the GLBR comprised less than 3 percent of the total clone distribution.

Table 7. Ribosomal DNA clone distribution in a hydrogen-enhanced, gas lift, bioreactor (GLBR) treating ammonium perchlorate.

Clone	Preliminary classification[a]	GenBank match[b]	Clone	
			Freq[c]	%
UI-10	Bacteria/unclassified	94%, uncultured bacterium; ZZ-S4E7; EF613425	1	1.2
UI-15	Bacteroidales/unclassified	99%, uncultured bacterium; B103; EF029344	1	1.2
UI-29	Erysipelotrichaceae/unclassified	97%, uncultured bacterium; Fin_CL-030746_OTU-2; EU808349	1	1.2
UI-30	Anaerolineae/unclassified	97%, uncultured bacterium; SHA-31; AJ306745	1	1.2
UI-31	Spirochaetaceae/unclassified	98%, uncultured bacterium; MR45; DQ661709	1	1.2
UI-32	Lentisphaerae/unclassified	90%, uncultured bacterium; 362; AJ536842	1	1.2
UI-33	Bacteria/unclassified	99%, uncultured Verrucomicrobia bacterium; MN079; AM157507	1	1.2
UI-34	Intrasporangiaceae/Unclassified	100%, uncultured Intrasporangiaceae bacterium; ska19; AY710281	1	1.2
UI-35	Thermotogaceae/Unclassified	98%, uncultured bacterium; R3ENDE1; DQ401516	1	1.2
UI-36	Opitutus	90%, uncultured Verrucomicrobia bacterium; BBD_216_46; DQ446121	1	1.2
UI-37	*Bacteroidetes*/Unclassified	95%, uncultured bacterium; MR3; DQ661705	1	1.2
UI-38	Bacteria/unclassified	99%, uncultured bacterium; G2-7; AY280646	1	1.2
UI-39	Bacteroidales/unclassified	97%, uncultured anaerobic bacterium; B-1BG; AY953168	1	1.2
UI-40	*Clostridia*les/unclassified	98%, uncultured bacterium; biogas-DI-b78; DQ419704	1	1.2
UI-41	Bacteria/unclassified	92%, uncultured bacterium; ZZ-S4E7; EF613425	1	1.2
UI-42	*Bacteroidetes*/unclassified	95%, uncultured bacterium; TSAC21; AB186809	1	1.2
UI-43	Beta*Proteobacteria*/unclassified	96%, uncultured bacterium; CM12; EF580928	1	1.2
UI-44	*Clostridia* /unclassified	99%, uncultured bacterium; PLB02; AB232802	1	1.2
UI-45	Sphingobacteriales/unclassified	98%, uncultured *Bacteroidetes* bacterium; AKYG1727; AY921801	1	1.2
UI-46	Dechloromonas	100%, *Dechloromonas sp.* JM; AF323489	47	55.3
UI-47	Ancalomicrobium	98%, *Rhizobiales* bacterium TP249; EF636050	3	3.5
UI-48	Bacteroidales/unclassified	99%, uncultured bacterium; B103; EF029344	1	1.2
UI-49	Bacteria/unclassified	94%, uncultured bacterium; 4E1_cons; EF688196	4	4.7
UI-50	Smithella	99%, uncultured bacterium UASB_TL9; AF254389	2	2.4
UI-51	Trichococcus	99%, uncultured bacterium; TSAT08; AB186876	1	1.2
UI-52	Bacteria/unclassified	94%, uncultured bacterium; 5C77; DQ663980	1	1.2
UI-53	Caldilineacea/unclassified	98%, uncultured bacterium; D30-814; EF174253	2	2.4
UI-54	Cellulomonas	92%, uncultured Cellulomonadaceae bacterium; HT06Ba07; EU016433	2	2.4
UI-55	Sedimentibacter	96%, uncultured bacterium; ATB-KM1254; DQ390276	3	3.5
UI-56	Bacteroidales/unclassified	99%, uncultured bacterium; G-5; DQ443931	1	1.2
UI-57	Bacteroidales/unclassified	100%, uncultured bacterium; 30a11; EF515551	1	1.2

[a] Determined with RDP Classifier program using naïve Bayesian rRNA classifier (Wang et al. 2007).

[b] GenBank sequences most closely related to the 16S rDNA clone sequences. Accession numbers and sequence identities based on maximum BLAST scores are listed.

[c] Frequency of clones out of a total of 85 after 180 days of ammonium perchlorate treatment.

MCAAP GAC-FBR treating TNT, TNB, and RDX clone distribution

Cloning the community 16S rRNA genes of the GAC-FBR treating TNT, TNB, and RDX revealed the 21 unique species representing eight distinct phyla (Table 8). The reactor was found to be enriched in *Chloroflexi*, *Proteobacteria*, and *Firmicutes* at 37, 27 and 12 percent of the total bacterial population, respectively.

Table 8. Bacterial community phylogenetic distribution in a pilot-scale granulated activated carbon-fluidized bed reactor (GAC-FBR) treating hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) 1,3,5-trinitrobenzene (TNB) and 2,4,6-trinitrotoluene (TNT) based on 16S rDNA clone distribution.

Phylum[a]	Sp.[b]	Clone (%)[c]
Acidobacteria	1	1.1
Actinobacteria	1	8.7
Bacteroidetes	2	3.3
Chloroflexi	2	37.0
Firmicutes	3	12.0
Fusobacteria	1	1.1
Nitrospira	3	4.3
Proteobacteria	4	27.2
unclassified bacteria[d]	4	5.4

[a] Determined with the RDP *Classifier* program using naïve Bayesian rRNA classifier (Wang et al. 2007).
[b] Number of unique species within the phylum.
[c] Percent of clones belonging to the phylum out of a total of 92 successfully sequenced clones.
[d] Bacterial sequences with no close matches within the RDP.

Additionally, there was a substantial *Acidobacteria* population, which made up approximately 9 percent of the total clone distribution. The most abundant species within the bioreactor was a species of *Anaerolinea* (clone M-8) (Table 9). The clone made up 21 percent of the bacterial population within the reactor and was found to be most closely related to uncultured bacterium SHA-147. The second most abundant bacterium was an unclassified Anaerolineae species (clone M-9), which consisted of 16 percent of the population. Clone M-9 had a 96 percent ribosomal sequence identity to *Chloroflexi* bacterium AKYG493. Collectively, clones M-8 and M-9 represented the total *Chloroflexi* population and made up 37 percent of the clone distribution. Substantial *Geobacter* (clone M-12) and *Desulfovibrio* (clone M-13) populations were also identified composing 13 and 12 percent of the total population respectively.

The *Geobacter* was found to be 98 percent similar to uncultured bacterium clone JG135 and the *Desulfovibrio* species had a 98 percent sequence similarity to uncultured bacterium clone R2b32. Collectively, these two clones made up 25 percent of the *Proteobacteria* population. Additionally, *Veillonellaceae* (clones M-11 and M-14) and Coriobacteriaceae (clone M-10) species were also found in significant numbers. The Coriobacteriaceae bacterium made up 9 percent of the population, and collectively the *Veillonellaceae* population made up 11 percent of the total population. All remaining clones comprised less than 3 percent of the total clone population.

Table 9. Ribosomal DNA clone distribution in a pilot-scale granulated activated carbon fluidized bed reactor (GAC-FBR) treating 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 1,3,5-trinitrobenzene (TNB).

Clone	Preliminary classification[a]	GenBank match[b]	Clone	
			Freq[c]	%
M-1	Syntrophobacterales/unclassified	99%, uncultured delta proteobacterium; MN033; AM157477	1	1.1
M-2	Gp3	99%, uncultured bacterium; PS-Ba63; EU399666	1	1.1
M-3	Ruminococcaceae/unclassified	97%, uncultured bacterium; RL247_aaj21f02; DQ799903	1	1.1
M-4	Oligotropha	87%, Rhodopseudomonas palustris CGA009; BX572608	1	1.1
M-5	Magnetobacterium	100%, uncultured bacterium; EA01; EU334523	1	1.1
M-6	Bacteria/unclassified	87%, uncultured bacterium; TTMF57; AY741701	1	1.1
M-7	Cetobacterium	99%, uncultured *Fusobacteria* bacterium; 2240; DQ409978	1	1.1
M-8	Anaerolinea	91%, uncultured bacterium; SHA-147; AJ306749	19	20.7
M-9	Anaerolineae/unclassified	96%, uncultured *Chloroflexi* bacterium; AKYG493; AY921883	15	16.3
M-10	Coriobacteriaceae/unclassified	99%, uncultured bacterium; ORSATC_g07; EF393140	8	8.7
M-11	*Veillonellaceae*/unclassified	94%, uncultured bacterium; RB90-49; AM159444	8	8.7
M-12	Geobacter	98%, uncultured bacterium; JG135; DQ138957	12	13.0
M-13	Desulfovibrio	98%, uncultured bacterium; R2b32; AF482438	11	12.0
M-14	*Veillonellaceae*/unclassified	98%, uncultured bacterium; E51; EU864464	2	2.2
M-15	Magnetobacterium	99%, uncultured bacterium; CLONG6; DQ478746	2	2.2
M-16	Magnetobacterium	100%, uncultured bacterium DGGE band 5; AJ238368	1	1.1
M-17	Bacteria/unclassified	99%, uncultured bacterium; SHA-41; AJ306787	2	2.2
M-18	Bacteroidales/unclassified	99%, uncultured anaerobic bacterium; A-2C; AY953218	2	2.2
M-19	Bacteroidales/unclassified	100%, uncultured bacterium; c5LKS78; AM086147	1	1.1
M-20	Bacteria/unclassified	97%, uncultured bacterium; SS-75; AY945880	1	1.1
M-21	Bacteria/unclassified	100%, unidentified bacterium; MUG24; AB011316	1	1.1

[a] Determined with RDP Classifier program using naïve Bayesian rRNA classifier (Wang et al. 2007).
[b] GenBank sequences most closely related to the 16S rDNA clone sequences. Accession numbers and sequence identities based on maximum BLAST scores are listed.
[C] Frequency of clones out of a total of 92 after 2 years of high explosives treatment.

4 Discussion

AFBR treating DNAN/MNA

Published accounts of microbial diversity within bioreactors treating nitramines are limited. Adrian et al. (2001) characterized the microbial communities of AFBR treating TNT using oligonucleotide probes and found that *Proteobacteria* populations increased as the TNT loading concentrations were increased, which implied microbial involvement in TNT removal. Kimura et al. (2003) studied the microbial community in a 2,4-DNP digesting bioreactor and based on 16S rDNA distribution, established that the culture was enriched in both *Actinobacteria* and *Proteobacteria*, suggesting involvement of both phyla in 2,4-DNP degradation.

Although the characterization of explosive degrading bioreactors is limited, several studies have characterized energetic-degrading microbial cultures. Using phospholipid fatty acid (PFLA) and terminal restriction fragment length polymorphism (T-RFLP) profiles Ringelberg et al. (2005) found that soil microbial communities were enriched in *Clostridium*, *Rhodococcus*, and *Beijerinckia* species in the presence of aqueous RDX. Each genus is known to harbor nitramine-degrading species (Bhuchan et al. 2003; Seth-Smith et al. 2002), demonstrating that *Firmicutes* in addition to *Proteobacteria* are likely important members of explosive-degrading communities.

Arnett et al. (2009) has also shown the predominance of *Proteobacteria* in an explosive-degrading culture, which originated from a munitions wastewater treatment plant. Cloning of the community 16S rRNA genes revealed that, when grown under homoacetogenic conditions, the culture consisted primarily of a *Geobacter sp.* and an *Acetobacterium sp.* When sulfate was added as an electron acceptor the culture became enriched with *Clostridiaceae*, *Sulfuricurvum*, and *Desulfovibrio* species, which in turn stimulated explosives degradation. Additional studies with nitramine-degrading cultures have identified several species of acetogens (Adrian and Arnett 2004; Sherburne et al. 2005), *clostridia* (Regan and Crawford 1994; Zhao et al. 2003a, 2003b) and *Enterobacteriaceae* (Kitts et al. 1994; Young et al. 1997; Zhao et al. 2002) that all play a role in energetic transformation under selective conditions. Collectively, these studies advocate that microbial diversity varies greatly within energetic-degrading microbial com-

munities. Nonetheless, enriched *Proteobacteria* populations are most frequently identified, which implies nitramine degradation may be a common trait among the phylum.

The addition of IM to the AFBRs clearly had a significant effect on the microbial population dynamics. Despite literature contentions, the *Proteobacteria* population did not proliferate, nor did a substantial *Firmicutes* population develop during the treatment period. Before the addition of IM, each reactor was dominated by a single species of *Desulfuromonales* belonging to the phylum *Proteobacteria* that grouped in the family *Geobacteraceae*. Several *Geobacteraceae* are known to degrade cyclic nitramines (Kwon and Finneran 2006, 2008). However, over the 60-day treatment period the *Desulfuromonales* population decreased significantly. In the presence of DNAN, the total *Proteobacteria* population decreased from 76 percent of the clone distribution to 6 percent. In the MNA amended reactor, the population decreased from 84 to 12 percent, and in the DNAN/MNA reactor the population declined from 79 to 16 percent. This does not preclude the organism's involvement in DNAN and MNA degradation, but does demonstrate that IM clearly had a negative affect on the growth of the population.

After the 60-day treatment period several unclassified species of bacteria were identified, including clone UC-34. The clone was not detected in AFBR #1 before the addition of DNAN to the system. However, after IM treatment, the UC-34 made up roughly one third of the total bacteria population. The clone was not detected in the MNA-amended reactor and only 1 percent of the clone distribution was made up of UC-34 in the DNAN/MNA-amended reactor. This suggested that MNA had an inhibitory effect on the bacterium. A BLAST search revealed that the clone was most closely related to a *Chlorobi* bacterium. Members of the phylum *Chlorobi* are obligate photolithothotrops that possess very limited versatility with regards to degrading organic compounds (Trüper 1981). Thus, the species was not believed to be directly involved in DNAN degradation.

The presence of IM enhanced the *Chloroflexi* population significantly in each reactor system. In AFBR #1, the population increased greater than forty-fold. The populations increased nearly four-fold in AFBR #2 and three-fold in AFBR #3. Specifically, the *Levilinea* (clone UC-14) population increased to 41 percent of the clone distribution in the presence of DNAN, 36 percent in the presence of MNA, and 28 percent in the presence of DNAN/MNA. Our data showed a marked increase in the *Levilinea sp*.

population following exposure to DNAN and MNA. Species of *Chloroflexi* are commonly found in wastewater treatment plants and are known to possess versatile metabolic traits (Björnsson et al. 2002; Kragelund et al. 2007). Kittelmann and Friedrich (2008) have demonstrated the ability of several filamentous *Chloroflexi* to dechlorinate perchloroethene. The phyla have also been associated with the reductive dehalogenation of polychlorinated biphenyls and pentachlorobenzene (Santoh et al. 2006; Watts et al. 2005). Additionally *Chloroflexi* have been detected in bioreactors used to treat 4-methylebenzoate and terephthalate (Wu et al. 2001a, 2001b). This generalized metabolic diversity in addition to the substantial increase in the *Levilinea* population indicated *Chloroflexi* as being involved in DNAN and MNA reduction.

GLBR treating perchlorate

Comparison of the raw sewage and the hydrogen-enhanced GLBR after 180 days of perchlorate treatment also revealed a significant change in population dynamics. The raw sewage was dominated by *Bacteroidetes*, *Firmicutes*, *Chloroflexi*, and a group of unidentified bacteria. One tenth of the population consisted of three *Proteobacteria*. However, in the GLBR each of *Bacteroidetes*, *Firmicutes*, *Chloroflexi*, and unidentified bacteria populations were approximately one third that of the inoculum. The GLBR was dominated by *Proteobacteria* at greater than 62 percent of the total population. Specifically, 55 percent of the clone distribution was made up of a single *Proteobacteria* species, which was identified as *Dechloromonas sp.* strain JM (clone UI-46). The most abundant clone in the raw sewage was UI-14 (unclassified Bacteroidales) at 14 percent of the total population. Interestingly, clone UI-46 was not observed in the raw sewage and UI-14 was not detected within the GLBR. Only two clones (UI-10 and UI-15) were identified in the inoculum and the GLBR suggesting the raw sewage had greater diversity than originally perceived and that the population readily adapted to the presence of hydrogen as a source of electrons and perchlorate as a potential terminal electron acceptor.

Strain JM was originally isolated from a bioreactor treating perchlorate contaminated groundwater (Miller and Logan, 2000). The strain was capable of reducing perchlorate in the presence of hydrogen; however, the organism was found to require an organic substrate for growth. No significant carbon source was added to the GLBR, which initially suggested a symbiotic relationship between UI-46 and one or more additional bacteria (Giblin et al. 2000). However, no significant fermentative population was identified within the reactor that could substantiate the prolific growth of

clone UI-46. Several autotrophic, hydrogen-utilizing, perchlorate-reducing *Dechloromonas* species have been isolated (Zhang et al. 2002; Shrout et al. 2005). It was hypothesized that clone UI-46 was in fact an autotrophic species, which was closely related to strain JM. Zhang et al. 2002 isolated a chemolithoautotrophic, perchlorate-reducing, *Dechloromonas* species (strain HZ), which had a 99.8 percent full-length 16S rRNA gene sequence similarity to strain JM. It is known that bacteria having ribosomal similarities of greater than 99 percent can express genomic variations in excess of 70 percent (Stackebant and Gobel, 1994). Because only one third (426 bp) of the ribosomal gene was sequenced, it was possible that clone UI-46 was a variant strain of JM, which was capable of autotrophic growth. Due to the predominance of the *Dechloromonas sp.* and its close relationship to strains JM and HZ, it was believed to be the organism directly responsible for perchlorate degradation within the hydrogen enhanced GLBR.

Hydrogen has been shown to support explosives degradation under anaerobic conditions (Adrian et al. 2003), and several hydrogen-utilizing species of *Acetobacterium* (Adrian and Arnett, 2004; Sherburne et al. 2005), and *Desulfovibrio* (Arnett and Adrian, 2009) have been identified. However, neither of these genuses were identified in the GLBR, and *Dechloromonas* are not known to degrade nitrated compounds. Hence, it is not known how the bioreactor technology will respond to a commingled influent containing nitrated compounds. Future studies are focused on assessing the microbial population dynamics within the hydrogen enhanced GLBR treating both RDX and perchlorate simultaneously.

MCAAP GAC-FBR treating TNT, TNB, and RDX

One fourth of the evolved GAC-FBR population was made up of two *Proteobacteria* clones M-12 (*Geobacter sp.*) and clone M-13 (*Desulfovibrio sp.*). Several *Geobacter* species are known to degrade RDX via extracellular electron shuttling (Kwon and Finneran 2006). Arnett and Adrian (2009) isolated an RDX-degrading, chemolithotrophic, *Desulfovibrio sp.*, which was capable of growth on RDX as a sole source of carbon and nitrogen. Boopathy et al. (1998) has demonstrated the degradation of 1,3,5-trinitrobenzene (TNB), RDX, and octahydro-1,3,5,7-tranitro-1,3,5,7-tetraazocine (HMX) by a sulfate-reducing consortium consisting of strains of *D. desulfuricans*, *D. gigas* and *D. vulgaris*. Furthermore, Zhao et al. (2003b; 2004) have isolated several RDX-degrading *Desulfovibrio* species from both anaerobic sludge and marine sediments. The relative abundance of known explosive-degrading *Proteobacteria* within the GAC-FBR suggested their involvement in energetic transformation, which is consistent

with many literature reports (Adrian et al. 2001; Ringelberg et al. 2005; Arnett et al. 2009). However, the involvement of the *Chloroflexi* population in the degradation process could not be ruled out.

Data from the AFBRs suggested that DNAN and MNA may in fact inhibit *Proteobacteria* growth. This may or may not affect the bioreactors overall performance in treating influents containing DNAN, MNA, and high explosives such as RDX. The pilot-scale reactor was found to be enriched in two species of Anaerolineae (clones M-8 and M-9), both of which belonged to the phylum *Chloroflexi*. Collectively, these clones consisted of 37 percent of the total distribution within the reactor. Again, due to the diverse catabolic capabilities of *Chloroflexi*, it is likely that these clones may have also contributed to the degradation process. Therefore, if DNAN and MNA do in fact inhibit *Proteobacteria* populations, it is likely that the *Chloroflexi* would be enriched for, and that explosives degradation would be expected to continue.

No identifiable perchlorate-reducing population was identified within the GAC-FBR. However, Maloney et al. (2008a) demonstrated that the pilot-scale reactor was capable of removing perchlorate to below detection levels, but only when allowed to recirculate for an extended periods. This suggested an acclimation period was needed involving a shift in the microbial population. As with the GLBR, diversity was likely greater than initially perceived and, over time, a perchlorate-reducing population did evolve. Perchlorate and RDX have been shown to be degraded simultaneously in bench scale AFBRs that were fed ethanol as an electron donor (Atikovic et al. 2008, Maloney et al. 2008a, 2008b). The authors observed that degradation varied based on the amount of electron donor supplied. Additionally, it was found that reactors treating RDX and perchlorate were more efficient at transforming each of the compounds individually. This suggested that competition within the reactors for available electron donors likely had a substantial effect on the bacterial populations. Nonetheless, the experiments clearly demonstrated the ability of AFBRs to treat RDX and perchlorate commingled.

5 Conclusions

The 16S rRNA gene clone library results indicated that each reactor technology possessed very diverse population dynamics contingent on the explosive component being treated and the electron donor supplied. DNAN and MNA significantly impacted the microbial population dynamics in the AFBR. In the presence of DNAN and MNA, the *Proteobacteria* population (*Desulfuromonales sp.*) decreased significantly and a single *Chloroflexi* (*Levilinea sp.*) became the dominant organism in each reactor system. In the GAC-FBR, a substantial *Proteobacteria* population proliferated, suggesting that DNAN and MNA may inhibit growth of the phyla.

However, a substantial *Chloroflexi* population was also identified in the pilot-scale reactor. Due to the metabolic diversity associated with the phylum *Chloroflexi*, it is likely the population may have played an important roll in the degradation process. The GLBR was clearly enriched in *Proteobacteria* (*Dechloromonas sp.*), which was undoubtedly responsible for perchlorate reduction within the reactor. It is unclear how each reactor will perform with commingled IM formulations such as PAX-21, but it is likely that bioreactor technologies that stimulate both *Proteobacteria* and *Chloroflexi* populations may be necessary to meet future wastewater treatment needs.

Acronyms and Abbreviations

Term	Spellout
2,4-DNP	2,4-dinitrophenol
ABI	Applied Biosystems
AFBR	Anaerobic Fluidized Bed Reactor
BLAST	Basic Local Alignment Search Tool
BSA	Bovine Serum Albumin
CERL	Construction Engineering Research Laboratory
CN	Installations Division
CN-E	Environmental Processes Branch
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
DNAN	2,4-dinitroanisole
dNTPs	Deoxynucleoside Triphosphates
ESTCP	Environmental Security Technology Certification Program
ERDC	Engineering Research and Development Center
GAC	Granulated Activated Carbon
GLBR	Gas Lift Bioreactor
GAC-FBR	Granulated Activated Carbon-Fluidized Bed Reactor
HPLC	High Pressure Liquid Chromatography
HRT	Hydraulic Retention Time
MCAAP	McAlister Army Ammunition Plant
MNA	*n*-methyl-4-nitroaniline
PAX	Picatinny Arsenal eXplosive
PCR	Polymerase Chain Reaction
PLFA	Phospholipid Fatty Acid
rDNA	ribosomal Deoxyribonucleic Acid
RDP	Ribosomal Database Project
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
rRNA	ribosomal Ribonucleic Acid
Sp	Species
TNB	1,3,5-trinitrobenzene
TNT	2,4,6-trinitrotoluene
T-RFLP	Terminal Restriction Fragment Length Polymorphism

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
17-09-2009	Final	

4. TITLE AND SUBTITLE
Polymerase Chain Reaction (PCR) Analysis of Microbial Consortia in Wastewater Treatment Processes for High Explosives

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT

6. AUTHOR(S)
Clint M. Arnett, Giselle Rodriguez, and Stephen W. Maloney

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Engineer Research and Development Center (ERDC)
Construction Engineering Research Laboratory (CERL)
PO Box 9005,
Champaign, IL 61826-9005

8. PERFORMING ORGANIZATION REPORT NUMBER
ERDC/CERL TR-09-34

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Engineer Research and Development Center (ERDC)
Cold Regions Research and Engineering Laboratory (CRREL)
72 Lyme Road
Hanover, NH 03755-1290

10. SPONSOR/MONITOR'S ACRONYM(S)
CEERD-RR-N

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The engineering aspects of bioreactors treating wastewaters contaminated with high explosives are better understood than the microbial communities within the reactors. Five anaerobic bioreactors treating energetics were studied to better understand bacterial compositions in each reactor system as related to influents containing various energetic materials. Studied were: three anaerobic fluidized bed reactors (AFBR) treating DNAN and MNA; one gas lift bioreactor (GLBR) treating ammonium perchlorate; and one pilot-scale granulated activated carbon-fluidized bed reactor (GAC-FBR) treating TNT, RDX, and TNB. Bacterial profiles were derived by cloning and sequencing the total 16S rRNA genes from each reactor contaminant combination. In the presence of DNAN and MNA, the AFBRs communities showed bacteria of the phylum Chloroflexi represented 30 percent of the total clone distribution in each reactor. In the presence of ammonium perchlorate, the GLBR was composed primarily of Proteobacteria in excess of 60 percent of the clone distribution. The GAC-FBR treating TNT, RDX, and TNB was enriched in both Chloroflexi and Proteobacteria (37 and 27 percent of the total clone distribution, respectively). Assuming the most prevalent bacteria were enriched by the presence of the contaminant, the data indicated that Chloroflexi and Proteobacteria likely played an important roll in energetic degradation.

15. SUBJECT TERMS
wastewater treatment, insensitive munitions (IM), explosives, munitions waste disposal, hazardous waste management

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)
Unclassified	Unclassified	Unclassified	SAR	42	